Final Abstract Number: 55.031
Session: Diagnostics
Date: Saturday, June 16, 2012
Time: 12:45-14:15
Room: Poster & Exhibition Area

Serological-proteome analysis of the parasite *Schistosoma mansoni*

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Background: Schistosomiasis is an important parasitic disease, which affects more than 210 million people around the world. The control of schistosomiasis has been based on the use of the drug Praziquantel. While the use of the chemotherapy has an effect in the morbidity it does not prevent the re-infection. Therefore, the development of a long term protection based on vaccination is still an important priority. Another area that deserves attention is the search for new diagnostic candidates, where the deficiency of effective diagnostic assays has been related as one factor that contributes to transmission. The immunological mechanism that prevents the infection developed by individuals naturally resistant may indicate possible biomarker candidates. Genomic data for schistosomes have become increasingly available and post-genomic technologies have matured, proposing a more rational discovery of biomarkers.

Methods: *S. mansoni* adult worm protein extract were probed with sera of infected individuals (INF) and non-infected individuals from endemic area (normal endemic, NE) using two dimensional electrophoresis coupled to western blot (2D-WE), and the pattern of spots recognized compared. Immunoreactive proteins were identified by mass spectrometry. All immunoreactive proteins identified were expressed *in vitro* to be used in a protein microarray experiment. Two of the recombinant proteins were probed with the pooled sera with which they were originally identified.

Results: Immunoreactive profile showed that there is no correlation between the amount of adult worm protein expressed and their antigenicity pattern. Although both sera shared most of the immunoreactive proteins recognized, 8 protein spots reacted exclusively with the INF sera and 1 with the NE sera. A total of 47 different proteins were identified as immunoreactive and 27 of them were successfully expressed. Blot of two of the recombinant proteins confirmed the right identification of the immunogenic spot in the 2D-WE and also that the recombinant proteins maintained the immunogenic epitope present in the native protein.

Conclusion: The association of two-dimensional electrophoresis and western blot had enabled a pre-screening of immunoantigenic proteins of the parasite, while the microarray technique will refine the list of potential candidates for subsequent testing as protective or diagnostic antigens.

http://dx.doi.org/10.1016/j.ijid.2012.05.542

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Vis à vis microscopy and molecular biology for the malaria diagnosis

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Background: Accurate diagnosis of *Plasmodium* infections is crucial for morbidity and mortality reduction in tropical areas. Studies of epidemiology and immunity depend on accurate detection, diagnosis, and density estimation. Traditionally, malaria diagnosis has been done with light microscopy but nowadays molecular methods have become widely used.

Methods: In this study we compared: a) conventional microscopy by Giemsa-stained thick and thin blood film; b) semi-inested multiplex malaria PCR (SnM-PCR) described by Rubio *et al.* 1999 based on the amplification of the 18S small subunit ribosomal RNA gene; and c) real time PCR (qPCR) by commercial kits (PrimerDesignTMLtd). Blood of two hundred nineteen patients from Ethiopia were collected, stored and transported on filter paper for the PCR assay. Agreement between SnM-PCR and LM was determined by calculating Kappa Statistics with 95% confidence intervals.

Results: The sample analyzed by LM and SnM-PCR were positive in *Plasmodium* sp. 5.5% and 10.5% respectively. The agreement of SnM-PCR with other diagnostic methods was significant for the detection of *P. vivax*, but was low for the detection of *P. falciparum*. The concordance between both LM and SnM-PCR by the Kappa coefficient was good agreement (K = 0.62). The sensitivity of the LM was 52.5% and the sensitivity of qPCR was 70% when compared with the SnM-PCR (gold standard). The quantification by qPCR did not correlate with microscopic quantification for *P. vivax* samples (R2 = 0.652). The correlation for *P. falciparum* could not be assay due to poor detection of this specie by LM.

Conclusion: Molecular diagnostic is better than LM, in addition it be able to detect more positives samples which were missed by LM. Between both molecular methods, the SnM-PCR methodology was the most sensitive and specific method to detect both *Plasmodium* species from blood spots dried on filter paper. The real-time PCR method is suggested for malaria reference and research laboratories.

http://dx.doi.org/10.1016/j.ijid.2012.05.543